ON THE DEVELOPMENT OF *HETERODONTUS (CESTRACION) PHILLIPI.*

Part I.

By Professor William A. Haswell, M.A., D.Sc.

(Plates iv.-v.)

During a zoological expedition undertaken in September, 1893, for the purpose of exploring the marine zoology of Jervis Bay, a part of the New South Wales coast to the fauna of which little attention had been directed, I was struck by the unusually large number of the egg-shells of *Heterodontus (Cestracion)* which were to be seen on the beaches. Most of them were old and broken, usually covered with the tubes of *Eupomatus;* but after examining a number, I succeeded in obtaining a fresh one containing an egg with an early blastoderm. Eventually I found that many of these were to be found at low tide sticking in the crevices of the rocks, firmly wedged in by means of the spiral flange which forms such a remarkable feature of the egg-shell ; and with the help of of Mr. J. P. Hill, who was of the party, I succeeded in collecting a considerable number. Of these as many as possible were preserved in the camp, but a large number were taken whole to Sydney, and more satisfactorily dealt with in the laboratory: Mr. Hill, on a second trip to the same locality, brought back a good many more. Some of these were kept alive for days in the laboratory. The cutting of a door in the egg-shell did not seem to interfere with the development, and, with proper appliances for renewing or aërating the sea-water, I have little doubt that it would be possible in this way to follow the course of the development for a prolonged period. This I hope to be able to do with regard to the later stages at some future time. The present communication refers only to the stages prior to the appearance of the notochord: detailed reference to the literature is deferred. I am much indebted to my demonstrator, Mr. J. P. Hill, not only for help in obtaining the specimens, but also for preserving many of the blastoderms, for making measurements and indicating approximately the stage which each had reached, and for sectioning many of them.

Cestracion (Heterodontus) is a genus which is almost unique among the Elasmobranchs in having been represented by near relatives as far back as the Carboniferous period. Although the adult structure of the members of the family had even at that early period become highly specialised and widely divergent from the main line of Elasmobranch evolution, the hope is not an unreasonably sanguine one that the embryonic development of a type so ancient might exhibit some important primitive features. With regard to the stages now described, however, any expectations of the kind cannot be said to have been fulfilled; and what impresses one most in the results is the extraordinary persistency of certain characteristics which are not known to have any vital significance. There can be little doubt, for example, that the "orange spot" which forms such a striking feature of the egg of an Elasmobranch in its early stages, has been handed down with little change from Palæozoic times.

The blastoderm occupies a constant position in the egg. It is always situated much nearer the broader than the narrower end of the egg-shell. The extremity of the blastoderm destined to become posterior is always directed away from the broader end of the egg-shell. Balfour* states that in *Pristiurus* the blastoderm is similarly constant in its position near the rounded end of the egg, while in *Scyllium* it is always near the narrow end to which the shorter pair of filaments is attached.

The blastoderm, in its earlier stages, appears to the naked eye, as in other Elasmobranchs,† as a circular reddish orange spot, around which is a narrow light yellow band. When this orange spot has attained a diameter of about 2 mm. it assumes an oval shape, its

* Balfour, Development of Elasmobranch Fishes, p. 10.

† L.c. p. 10.

longer axis corresponding with the future long axis of the body. At its posterior end appears a crescentic dark area which has very much the appearance of a cleft passing right through the blastoderm, but which sections prove to be a cavity, the segmentation cavity, covered over by a thin transparent roof. As the blastoderm extends, this dark area becomes less strongly marked and gradually disappears.

The yolk is covered with a thin investment which is perfectly continuous with the non-nucleated protoplasmic network of the substance of the yolk, of which it is to be looked upon as a specially modified part.

The light yellow band referred to above extends more rapidly than the blastoderm, and soon forms a broad zone around the latter. As it extends its boundaries become more and more indistinct. This is due to the spreading out of the bed of fine-grained parablastic substance on which the blastoderm lies. A number of small rounded spots, which appear scattered over it, are found on the examination of sections to be produced by the development of rounded spaces or vacuoles.

The earliest specimen of which satisfactory sections were obtained (Fig. 1) is one in which the fine-grained bed of yolk extends beyond the edge of the blastoderm to rather more than half of the diameter of the latter. In this stage the blastoderm consists of a lenticular mass of chiefly rounded cells, resting directly on the fine-grained substance in the greater part of its extent, but becoming separated from the latter towards the posterior end by a small segmentation cavity. The most superficial layer of cells are closely packed together: they are irregular in size and shape, but form a tolerably definite layer. In the deeper strata the cells are more loosely arranged, with intercellular spaces. In these, as in the segmentation cavity, with which they are more or less directly continuous, there are irregular masses and strands of a finely granular material, which is strongly coloured by staining agents; frequently this matter adheres to the surface of the cells or the wall of the segmentation cavity so as to form a distinct investment: from its appearance and mode of occurrence this material is, without much doubt, of the nature of a coagulum formed as a result of the action of the fixing solutions on a fluid contained in the segmentation cavity.

Balfour (p. 53) remarks on the frequent presence of a membrane-like structure between the blastoderm and the yolk, readily affected by staining agents, and sets it down as a layer of coagulated albumen. Perenyi,* on the other hand, states that in *Torpedo marmorata*, the yolk is enclosed in a fine structureless membrane.

The mass of fine-grained substance contains a small number of nuclei. Its upper surface, forming the floor of the segmentation cavity, is raised up here and there into a rounded mass containing a nucleus. Continuous with the mass of parablast material which lies below the blastoderm is a thinner layer extending out some distance beyond the edge of the blastoderm. This is continuous with the posterior edge of the latter, and at this point contains several nuclei. Cell divisions at this stage seem to be going on somewhat slowly, as the majority of nuclei are in the resting stage.

In *Pristiurus*, to judge from Balfour's account, the segmentation cavity makes its appearance only at a considerably later stage. (Compare his figures 8 and 9, of Plate ii., and 1 of Plate iii.)

In the stages which immediately follow on that just described, though the blastoderm (Figs. 2 and 3) does not at first increase in size, the cells multiply by division so as to become much more numerous and smaller. The massive blastoderm becomes much thicker in front than behind. The segmentation cavity increases in extent, and forms in the middle a comparatively wide space covered over dorsally behind by a thin stratum formed by material which is transitional between the posterior portion of the blastoderm and the parablast. Cells soon cease to become formed from the parablast of the floor of the cavity; but a part

* "Beiträge zur Embryologie von *Torpedo marmorata*." Zool. Anz. ix. (1886).

of the parablast of its roof appears to divide into cells which are added to the posterior part of the blastoderm. A distinct upper layer of the blastoderm (" ectoderm" of various authors) is no longer recognisable.

C. K. Hoffmann* states that in *Acanthias*, at a stage in the development of the blastoderm which corresponds broadly with that just described, there is an invagination- or gastrula-cavity opening widely by a blastopore on the exterior. The mode of formation of this gastrula-cavity, he avers, is closely comparable to the gastrulation in Amphibia, Cyclostomi and Amphioxus. If we are to accept the statement that the cavity in question is a gastrula cavity, then necessarily we must admit the justness of the comparison with the corresponding cavities in other Chordates. Such an admission, however, would involve us in the greatest difficulties. For here we should have an invagination which is not connected with the formation of the archenteron or of the mesoderm or notochord, an invagination-cavity which virtually disappears before the first rudiment of the mesoderm has become differentiated. I do not think, however, that the statement of fact can be taken without confirmation, and am confident that more thorough investigation will show that *Acanthias* does not depart so widely from other Elasmobranchs in such an essential phase of its development. I have several series of sections of blastoderms of *Heterodontus* at or about the stage represented in fig. 3. These, so far as they were examined in the fresh state, all presented the appearance described by Hoffmann, an appearance seeming to indicate the presence of an open cavity below the posterior edge of the blastoderm. In one of them only does the cavity open on the exterior; and in this the opening is readily seen on a careful examination to have resulted from a rupture of the delicate roof of the cavity, most probably during the removal of the blastoderm from the egg.

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* "Beiträge zur Entwickelungsgeschichte der Selachii," Morph. J.B. 1896.

In brief, I contend that the cavity represented in my figs. 1-4 is in all cases the same thing, viz., the segmentation-cavity, and that Hoffmann's fig. 4, of Taf. ii. corresponds, or should correspond with my fig. 3. This is a phase which was apparently not represented in the specimens at Balfour's disposal.

The blastoderm now increases somewhat in diameter, though still remaining very thick anteriorly. The segmentation-cavity has somewhat increased in size, and extends under the entire blastoderm. At its posterior end, just below and somewhat in front of the posterior limit of the blastoderm, appears a collection of cells of irregular shape, some of which are evidently being formed from the parablast of the floor of the cavity. Only a very few similar cells occur further back. This accumulation of cells, which extends all round the posterior margin, forms the starting point in the formation of the parablast endoderm.

The next change of importance (Fig. 5) is the arching upwards of the posterior portion of the blastoderm, so that where it passes into the parablast it becomes for a short distance vertical, and soon inclined forwards, forming the embryonic rim, which extends round the entire posterior margin. At the same time the accumulation of cells at the posterior end greatly increases and becomes extended backwards as a thin layer (yolk endoderm) over the entire floor of the segmentation-cavity. These cells now send off processes which apparently join the processes of neighbouring cells, so that the whole comes to form a reticulum, in the meshes of which are to be recognised masses of the coagulum from the fluid of the segmentation-cavity. Similar cells extend backwards as a thin irregular layer immediately below the blastoderm in the roof of the segmentation-cavity. The blastoderm has now become considerably thicker, but still remains thickest towards the anterior end.

The embryonic rim now becomes more strongly inflected (Fig. 6), and the blastoderm becomes greatly extended anteriorly, at the same time becoming thinned out. The segmentation-cavity extends *pari passu* with the extension of the blastoderm, but becomes extremely shallow. The parablast endoderm extends over its floor, but, unless the constituent cells are united by long processes, cannot be said to form a continuous layer. There is every appearance that at this stage there is an active formation of parablast endoderm cells from the fine-grained parablast below the embryonic rim; and its substance soon becomes completely divided up into cells. Further forward a similar process goes on, though less actively. A change at the same time takes place in the form and arrangement of the cells of the blastoderm. In the neighbourhood of the embryonic rim they become vertically elongated, their arrangement approximating more and more to that of the cells of a columnar epithelium, while behind they remain more irregular in shape, and form a stratum several cells thick. It is of importance to observe that, whereas previous to the stage now reached all the cells of the blastoderm were filled with yolk-granules of the smaller size, the vertically-elongated cells now contain yolk only in their lower portions.

Balfour states that in *Pristiurus* at his stage B, *i.e.*, at the stage in which the involution to form the endoderm has just begun, the segmentation-cavity has completely disappeared, having become filled with an irregular network of cells.

The inflection of the blastoderm at the embryonic rim leads to the formation of a fold, the upper layer of which is ectoderm, the lower embryonic endoderm. The latter grows backwards along the entire posterior border of the blastoderm, but more rapidly along the middle line, the cavity below it giving rise to the archenteron (Fig. 7). As it extends backwards it apparently receives contributions of new cells from two sources; the greater number of the added cells are derived from the yolk-endoderm, but others are derived from the thin layer of cells which has been described above as lying below the roof of the segmentation-cavity. From the first the endoderm as it becomes formed assumes the character of an epithelium of vertically elongated cells.

The cavity below the endoderm (archenteron, gastrula-cavity) (Figs. 7 and 8, *ent.*) is a wide space which is bounded below only by the large-grained yolk with its protoplasmic network. Soon, however, its walls begin to curve inwards anteriorly, and eventually meet below so as completely to enclose the archenteric canal in its anterior portion—the enclosure gradually extending backwards.

EXPLANATION OF FIGURES.

ant., anterior end of blastoderm.

ect., ectoderm.

ect.[1], superficial layer of cells distinguishable before the comp'ction of segmentation.

end., endoderm.

end.[1], parablast endoderm.

ent., archenteron.

para., bed of fine-grained yolk with parablast nuclei.

Fig. 1.—Sagittal section of the blastoderm of *Heterodontus Phillipi* at a late stage of segmentation, showing the beginnings of the segmentation-cavity and the superficial layer.

Fig. 2.—Similar section of a somewhat later stage.

Fig. 3.—Stage with well-defined segmentation-cavity at the posterior end.

Fig. 4.—Somewhat later stage, in which the segmentation-cavity has become extended forwards and in which the first indication of the invagination is to be distinguished.

Fig. 5.—Later stage in which the involution has begun, and the rudiments of the parablast endoderm have become formed.

Fig. 6.—Stage in which the blastoderm has become considerably extended forwards and the parablast endoderm has become developed.

Fig. 7.—Similar section of a blastoderm in which the archenteron has become well established.

Fig. 8.—Transverse section of a blastoderm of a somewhat later stage, but before the first appearance of the notochord.